Bile Acids Are the Strong Indicators for the Progression of Metabolic Dysfunction Associated Steatotic Liver Disease in Ethnically Diverse Populations

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Background

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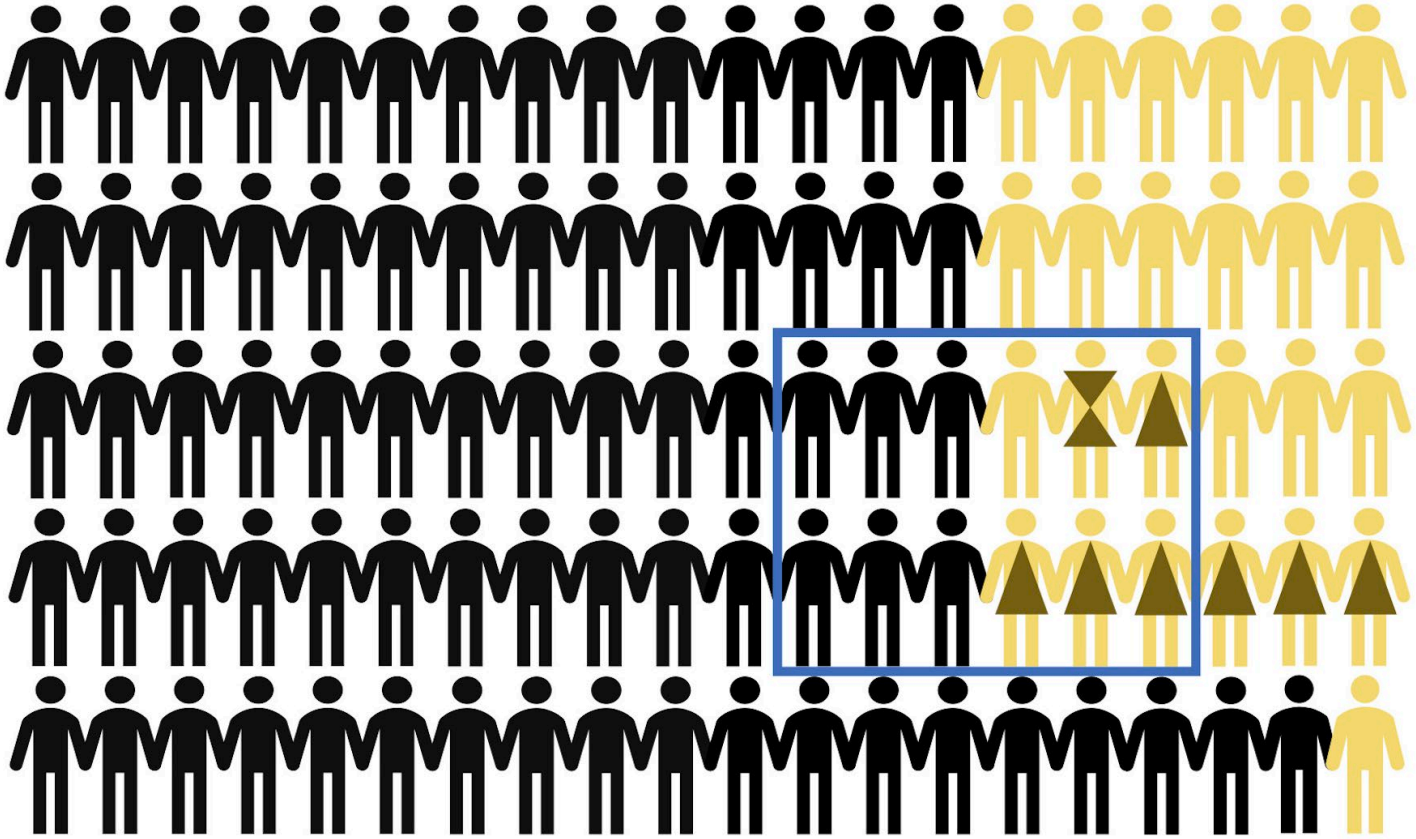
Metabolic dysfunction associated steatotic liver disease (MASLD) :a serious public health burden



North American population

25% affected by MASLD

30% progress to Metabolic associated steatohepatitis (MASH)

20% develop Fibrosis

Figure adopted from Barbosa and Lai, Hepatology Communications, 5(2), 2021.

Liver [illegible] and Metabolic Health

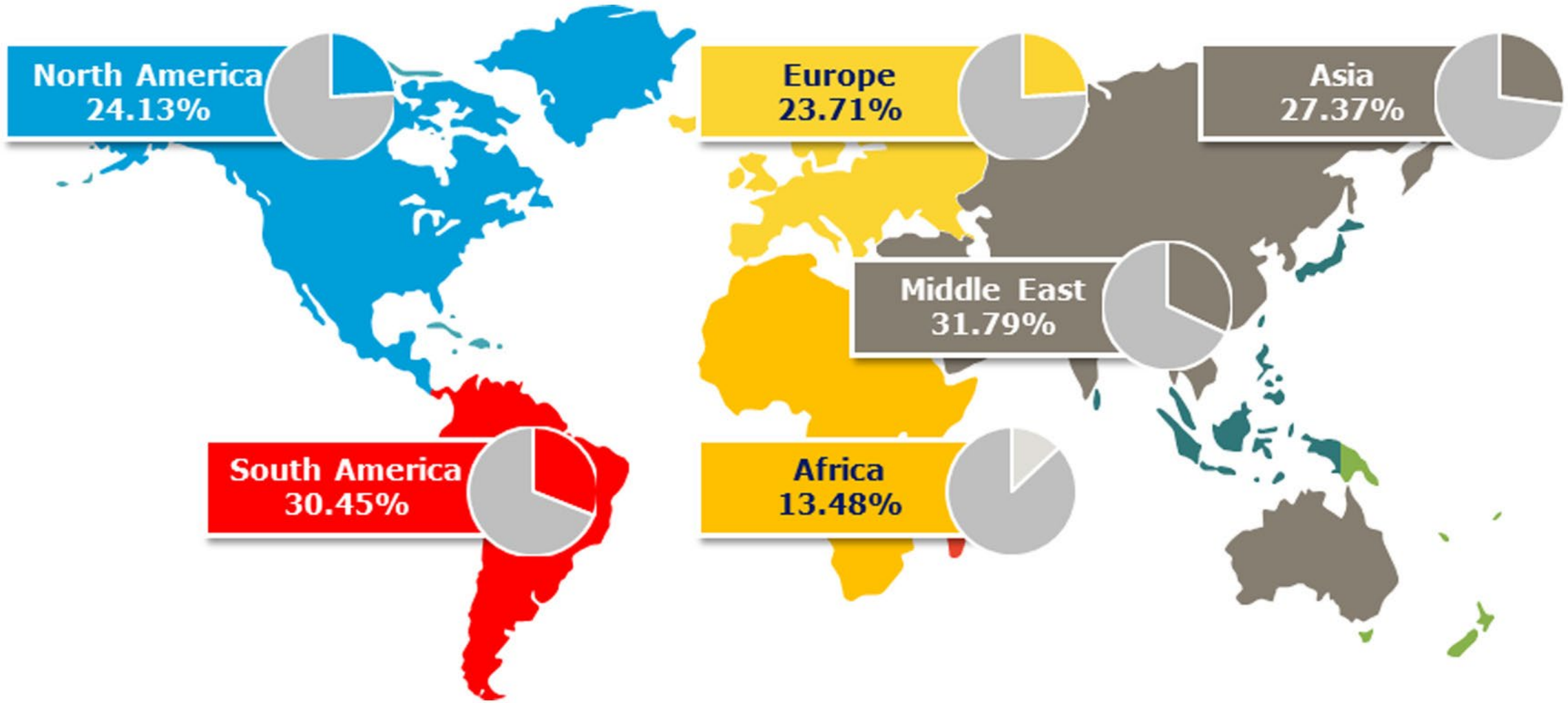


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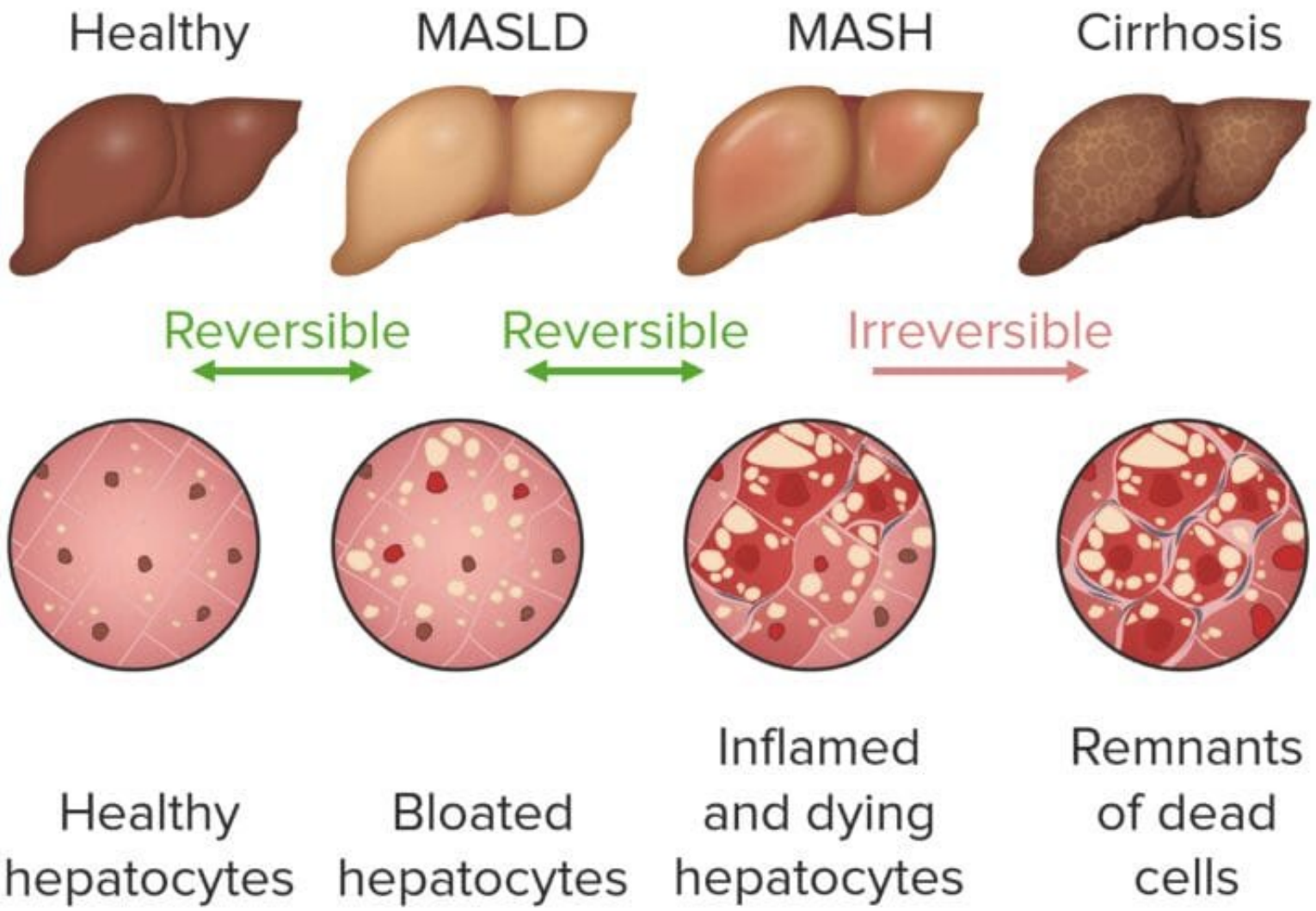
Figure adopted from Younossi et at, Hepatology, 69(6), 2019.

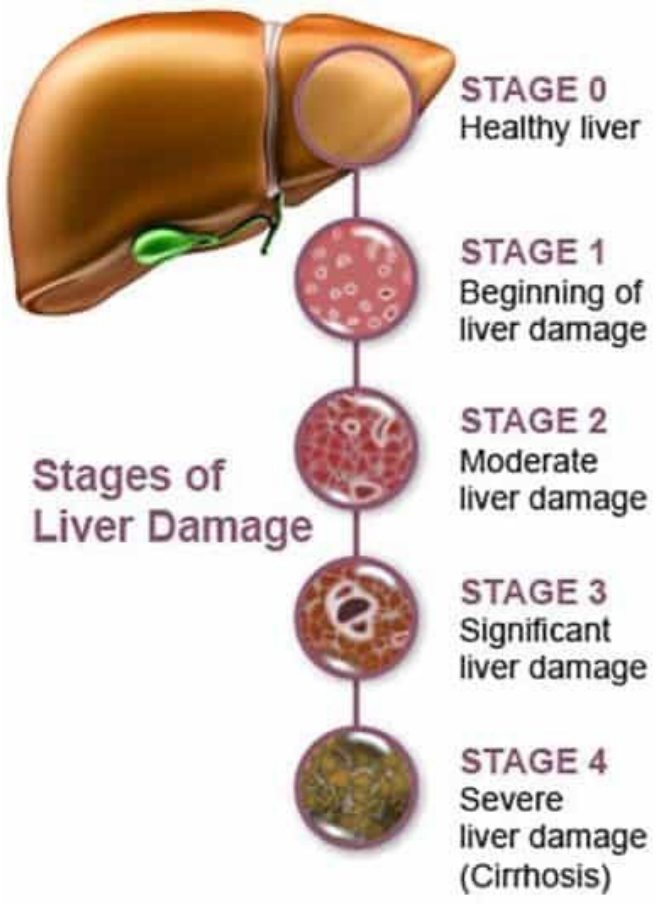
Progression of MASLD



Background

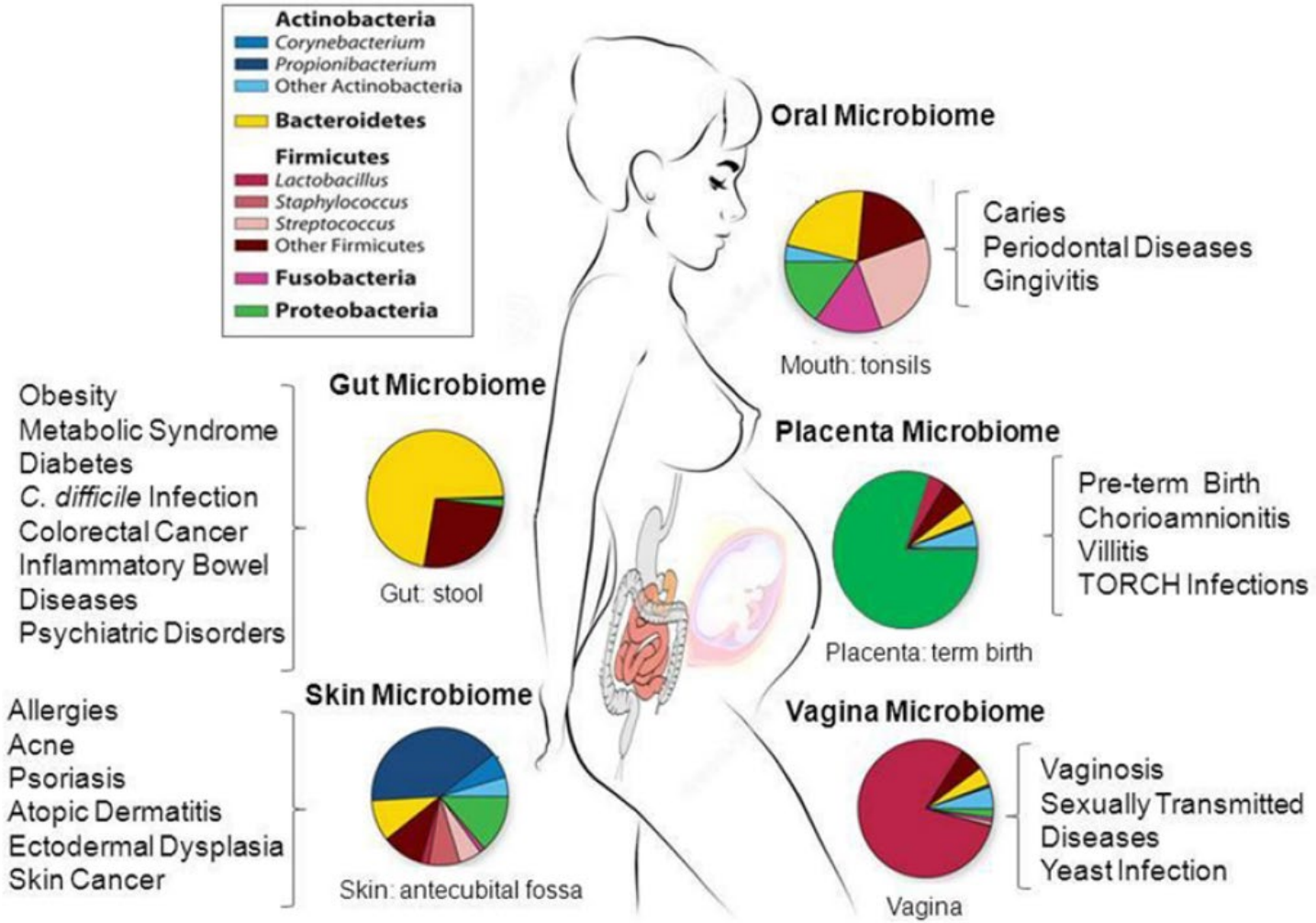
- Metabolic dysfunction associated steatotic liver disease (MASLD) is a major contributor to the burden of end-stage liver disease
- The mechanisms of MASLD progression to metabolic dysfunction associated steatohepatitis (MASH) cirrhosis and clinical decompensation are characterized by increasing fibrosis severity, but are not fully understood
- Bile acids are a large family of molecules that are synthesized from cholesterol in the liver and actively secreted along with cholesterol and phospholipids into the bile

Fibrosis progression



- Fibrosis stages 0-2 are early stages
  - The damage is often reversible with lifestyle changes and treatment of the underlying cause
- Fibrosis stages 3-4 are advanced stages
  - Stage 4 indicates a high risk of complications and death

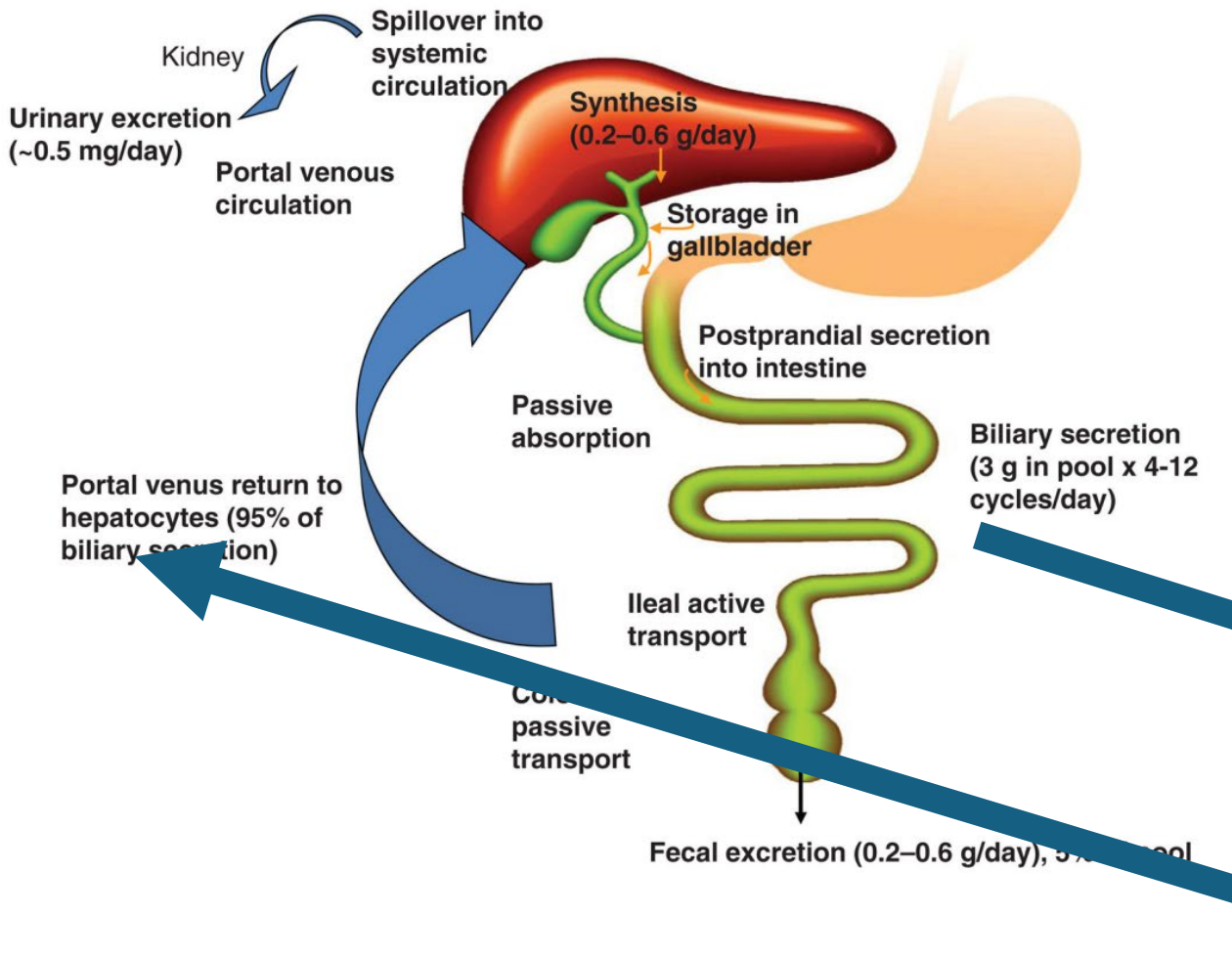
Human Microbiota Studies



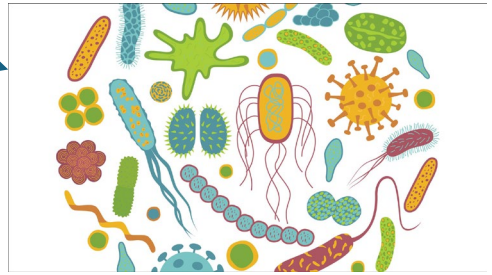
Source: Belizário and Napolitano (2015)

Microbiota in MASLD

- The intestinal microbiome has emerged as a key modulator of human health and disease
- Multiple studies noted changes in microbiome in patients with MASLD especially in those with advanced fibrosis
- They are however limited by
  - being cross-sectional and no data to relate changes in microbiome or its products to disease progression
  - being mainly correlational without mechanistic insight
- Bile acids are the key liver driver compounds that are
  - modified by intestinal microbiota and
  - have important biological activities that are relevant to MASLD



Primary bile acids



Secondary bile acids

Bile Acids with MASLD severity

- Serum bile acids are among most studied
  - Some studies examined fecal BA, but these are more rare
- Increased levels of total serum BA with worsening Fibrosis[1,2,11]
- Individual BA significant in F≥2 or F≥3

Increased in Fibrosis	GCDCA[2,7]	GCA[2,3,7,8,9]	7-Keto DCA[2]	GUDCA[2]	TCA[3,7]	TDCA[3,6,7]	DCA[3,5]	GDCA[3,8,9]	TDCA[3]	UCA[4]	βUCA[4]	7-HDCA[4]	Nor CA[4]	CA[5]	LCA[5,8]
Decreased in Fibrosis	CA[1]	CDCA[1]													

- Populations studied:
  - Western (US and Australia)
  - Hispanic (Guatemala and South Texas)
  - Asian (Chinese and Japanese)

Bile Acids with NAFLD severity

1. Caussy C, et al. Serum bile acid patterns are associated with the presence of NAFLD in twins, and dose-dependent changes with increase in fibrosis stage in patients with biopsy-proven NAFLD. Aliment Pharmacol Ther. 2019 Jan;49(2):183-193.
2. Nimer N, et al. Bile acids profile, histopathological indices and genetic variants for non-alcoholic fatty liver disease progression. Metabolism. 2021 Mar;116:154457.
3. Rivera-Andrade A, et al.. Circulating bile acid concentrations and non-alcoholic fatty liver disease in Guatemala. Aliment Pharmacol Ther. 2022 Jul;56(2):321-329.
4. Liu AN, et al. Secondary bile acids improve risk prediction for non-invasive identification of mild liver fibrosis in nonalcoholic fatty liver disease. Aliment Pharmacol Ther. 2023 Apr;57(8):872-885.
5. Kasai Y, et al.. Association of Serum and Fecal Bile Acid Patterns With Liver Fibrosis in Biopsy-Proven Nonalcoholic Fatty Liver Disease: An Observational Study. Clin Transl Gastroenterol. 2022 Jul 1;13(7):e00503.
6. Wegermann K, et al. Serum Bile Acid, Vitamin E, and Serotonin Metabolites Are Associated With Future Liver-Related Events in Nonalcoholic Fatty Liver Disease. Hepatol Commun. 2021 Jan 5;5(4):608-617.
7. Puri P, et al. The presence and severity of nonalcoholic steatohepatitis is associated with specific changes in circulating bile acids. Hepatology. 2018 Feb;67(2):534-548.
8. Kwan SY, et al. Bile Acid Changes Associated With Liver Fibrosis and Steatosis in the Mexican-American Population of South Texas. Hepatol Commun. 2020 Mar 19;4(4):555-568.
9. Adams LA, et al. Bile acids associate with specific gut microbiota, low-level alcohol consumption and liver fibrosis in patients with non-alcoholic fatty liver disease. Liver Int. 2020 Jun;40(6):1356-1365.

Comprehensive microbiome-bile acid relationship study aims

- To determine the relationship of fecal bile acids with fibrosis stage in a cross-sectional study of patients with MASLD
- To relate these changes to intestinal microbiome responsible for these changes in bile acids
- To confirm the linkage of fecal bile acid profile to severity of liver disease in an independent multi-ethnic cohort

Methods

Methods

- Study Cohort:
  - Pilot data VCU cohort of MASLD
- Validation cohort:
  - Multi-ethnic cohort: Türkiye, Brazil, Singapore
- Microbiome analysis:
  - 16S taxa
  - Meta transcriptome with focus on bile acid metabolizing genes
- Fecal bile acids:
  - VCU pilot data: Metabolon platform
  - Validation analysis: targeted bile acid targeted analysis (Dr. Huiping's lab at VCU)

Statistical Analysis (bile acids)

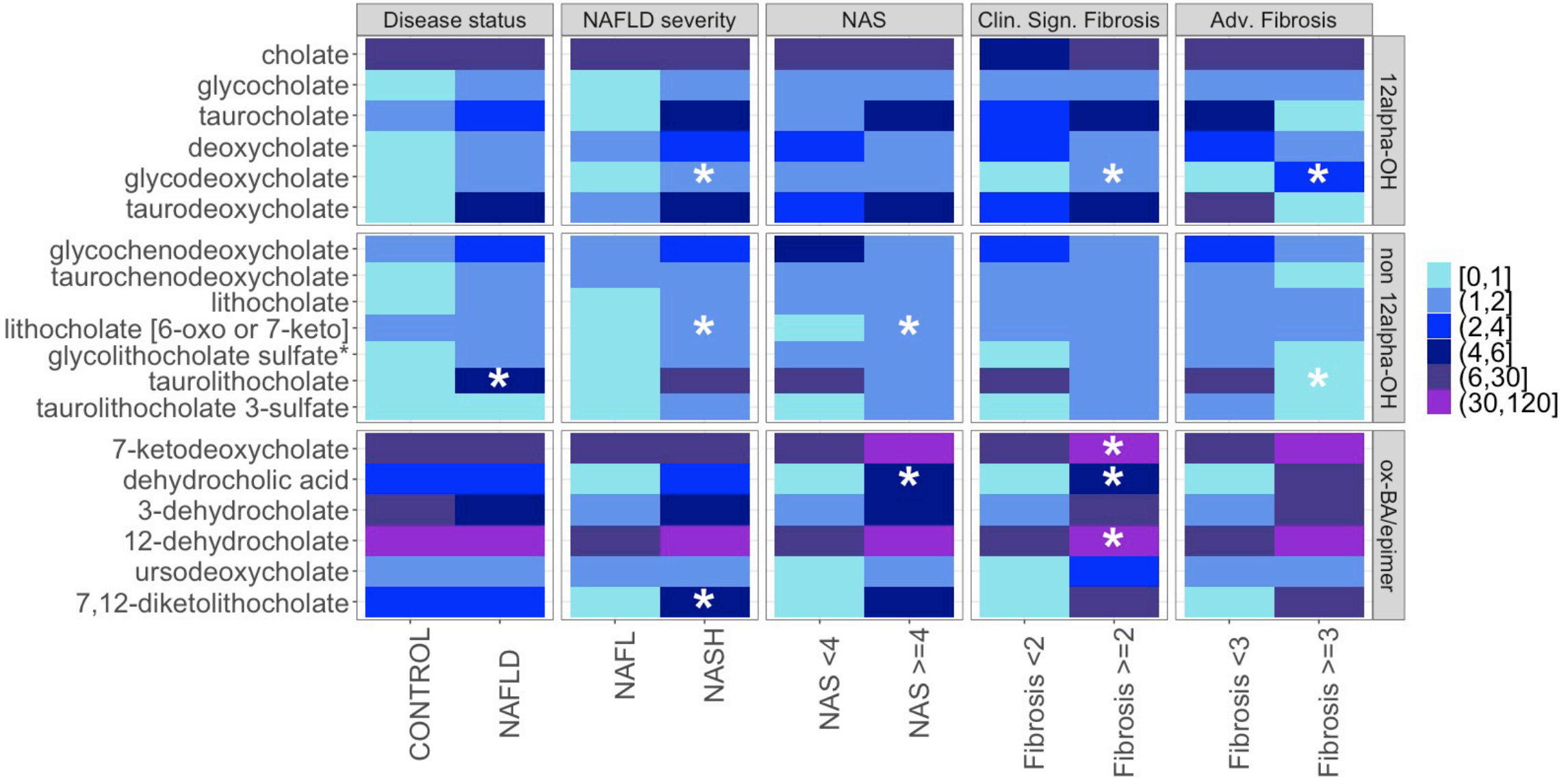
- Bile acid profiles in serum and stool were defined using mass spectrometry
- 3α-Hydroxysteroid dehydrogenase (hdhA) and bile acid inducible operon CD gene (baiCD) mRNAs were measured by qPCR and relative gene copy estimated
- DCA generation was evaluated in stool incubated under N_2 anaerobic conditions
- Statistical comparisons were performed with pairwise t-tests adjusted with Holm procedure for multiple comparisons correction.
- Significance was set at 0.05

Statistical Analysis (microbiome)

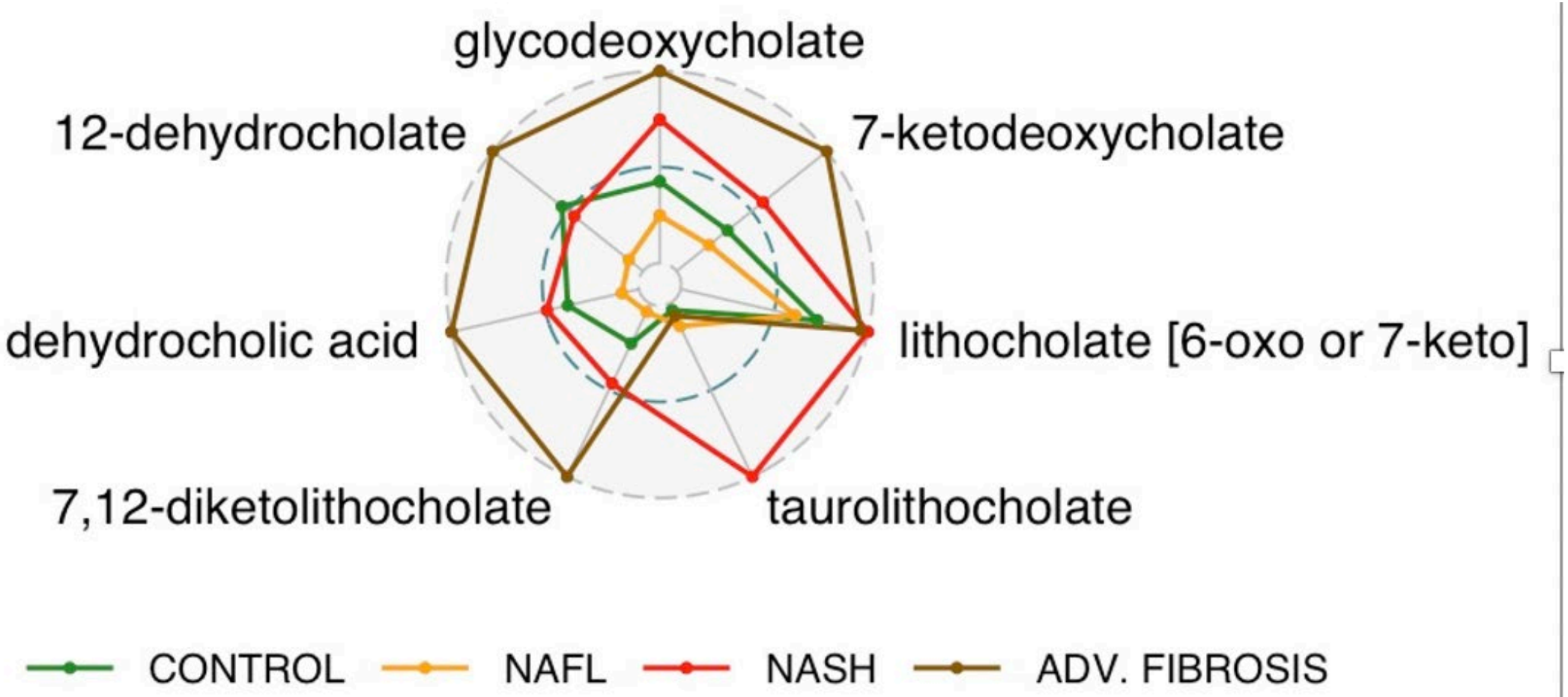
- Pilot data: VCU samples processed by Second Genome
- Validation data: Stool samples were collected at each site and shipped to VCU coordinating site for analysis
- 16S analysis was performed by SeqMatic NGS omics services simultaneously for all samples to minimize technical differences
- Differentially abundant taxa in advanced (stages <3 vs ≥3) fibrosis groups was identified using Linear Discriminant Analysis Effect Size (LEfSe) on the combined and site-stratified cohort LDA score ≥2 was set for significance

Results: pilot data

Bile acid profile in VCU cohort



Deoxycholate (DCA) and its derivatives are increasing with increasing fibrosis stage of MASH

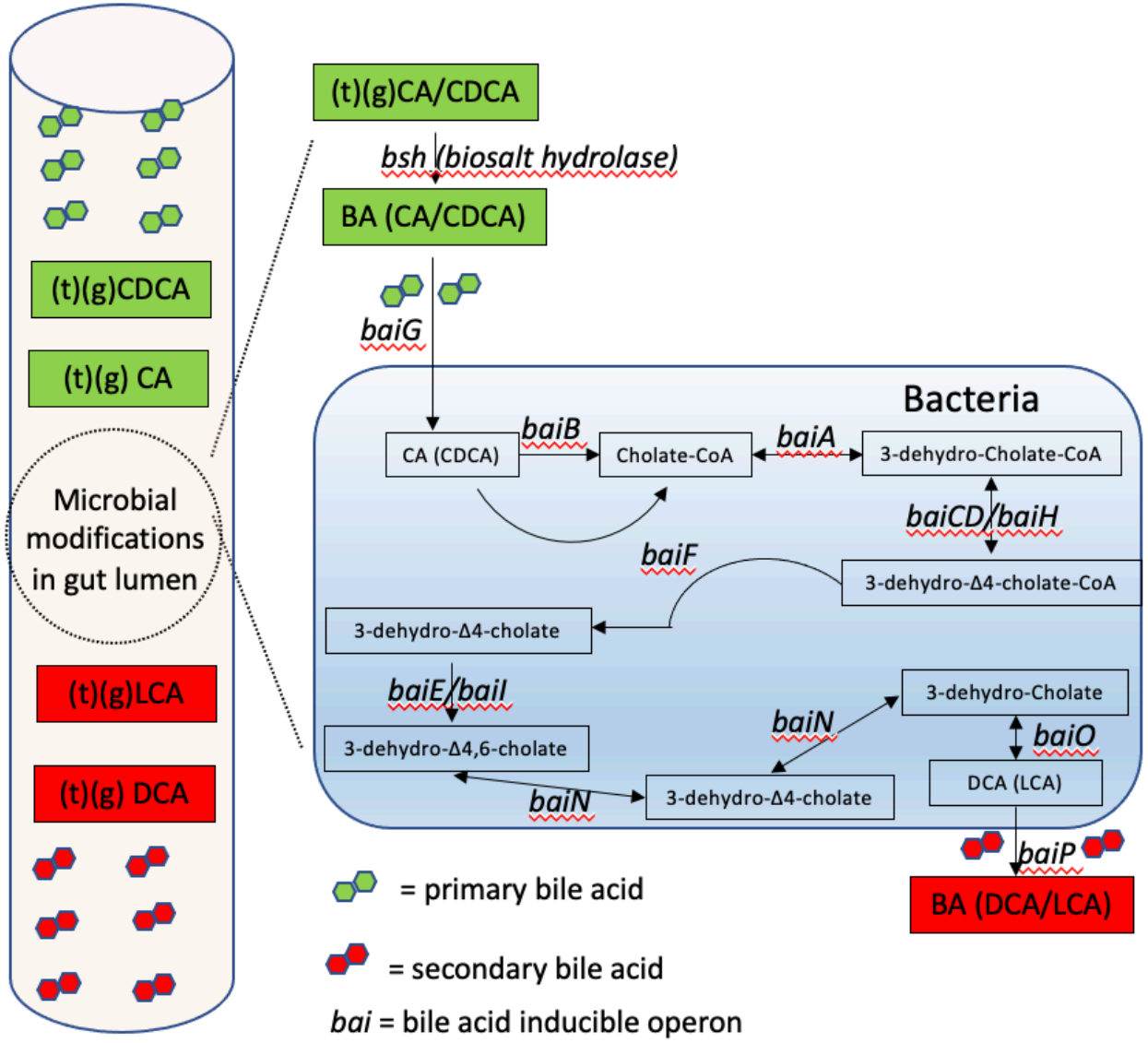


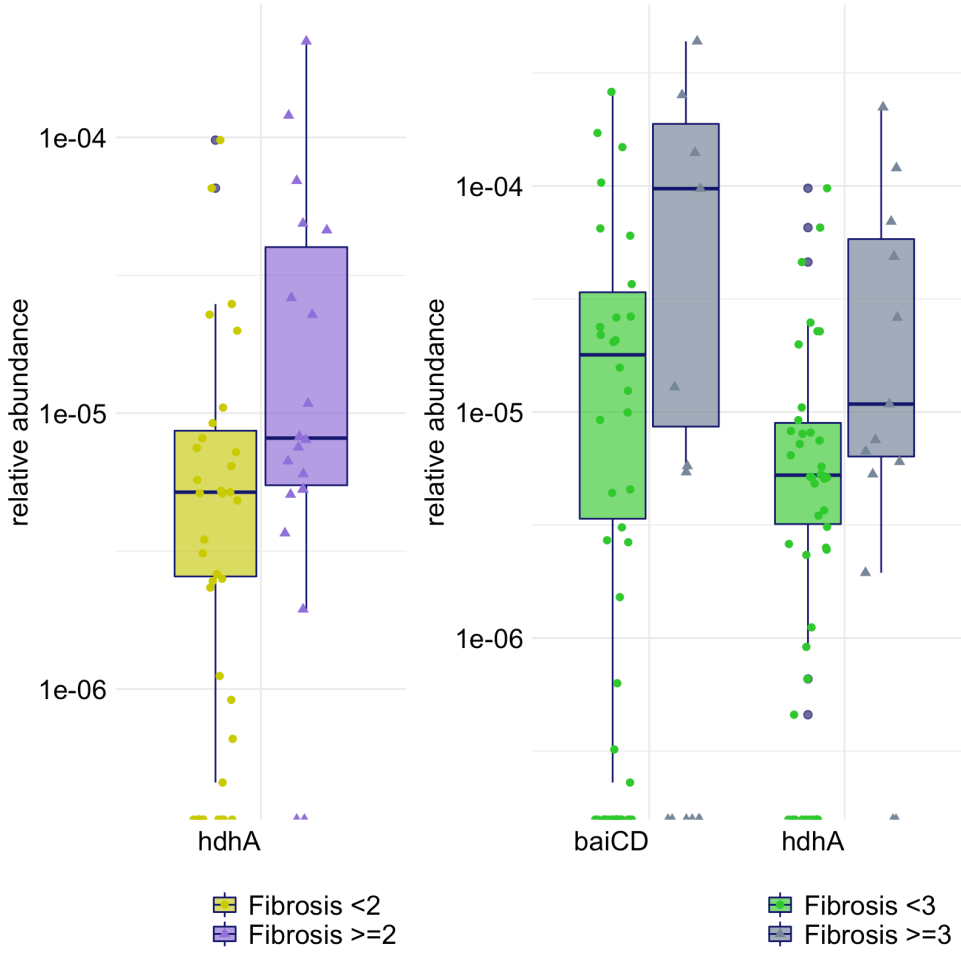
Stool BA significance in the context of current literature

- 5. Kasai Y, et al.. Association of Serum and Fecal Bile Acid Patterns With Liver Fibrosis in Biopsy-Proven Nonalcoholic Fatty Liver Disease: An Observational Study. Clin Transl Gastroenterol. 2022 Jul 1;13(7):e00503.
- 9. Adams LA, et al. Bile acids associate with specific gut microbiota, low-level alcohol consumption and liver fibrosis in patients with non-alcoholic fatty liver disease. Liver Int. 2020 Jun;40(6):1356-1365.
- 10. Smirnova et al. Metabolic reprogramming of the intestinal microbiome with functional bile acid changes underlie the development of NAFLD. Hepatology. 2022 Dec;76(6):1811-1824.

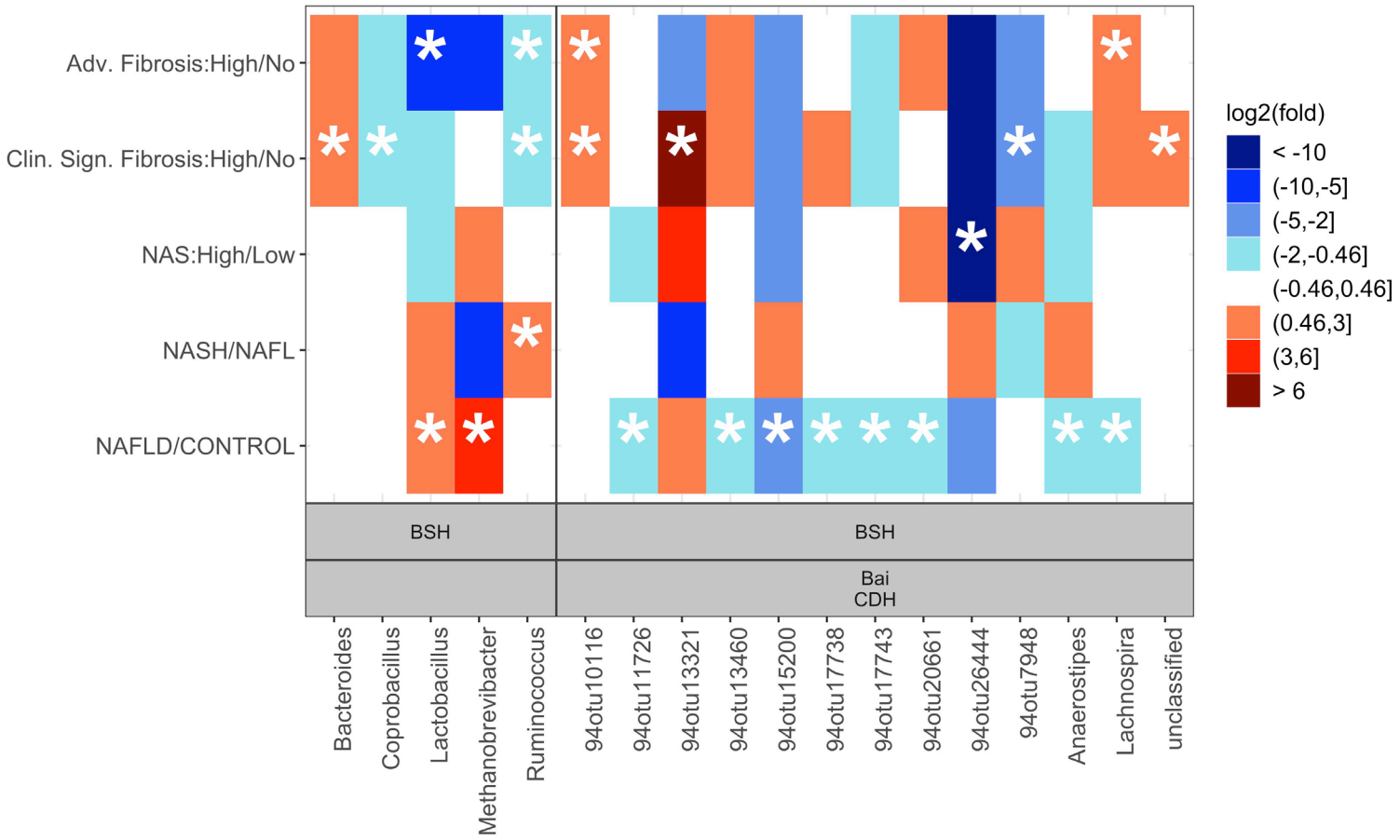
Increased in Fibrosis	GDCA[10]	7-Keto DCA[10]	DehydroCA[10]	12-DehydroCA[10]	CA[5]	CDCA[5]	UDCA[5]	DCA[9]	LCA[9]
Decreased in Fibrosis	TLCA[10]								

Mechanisms of increased DCA





Significant taxa known to contain genes involved in gut biotransformation of deoxycholate bile acid

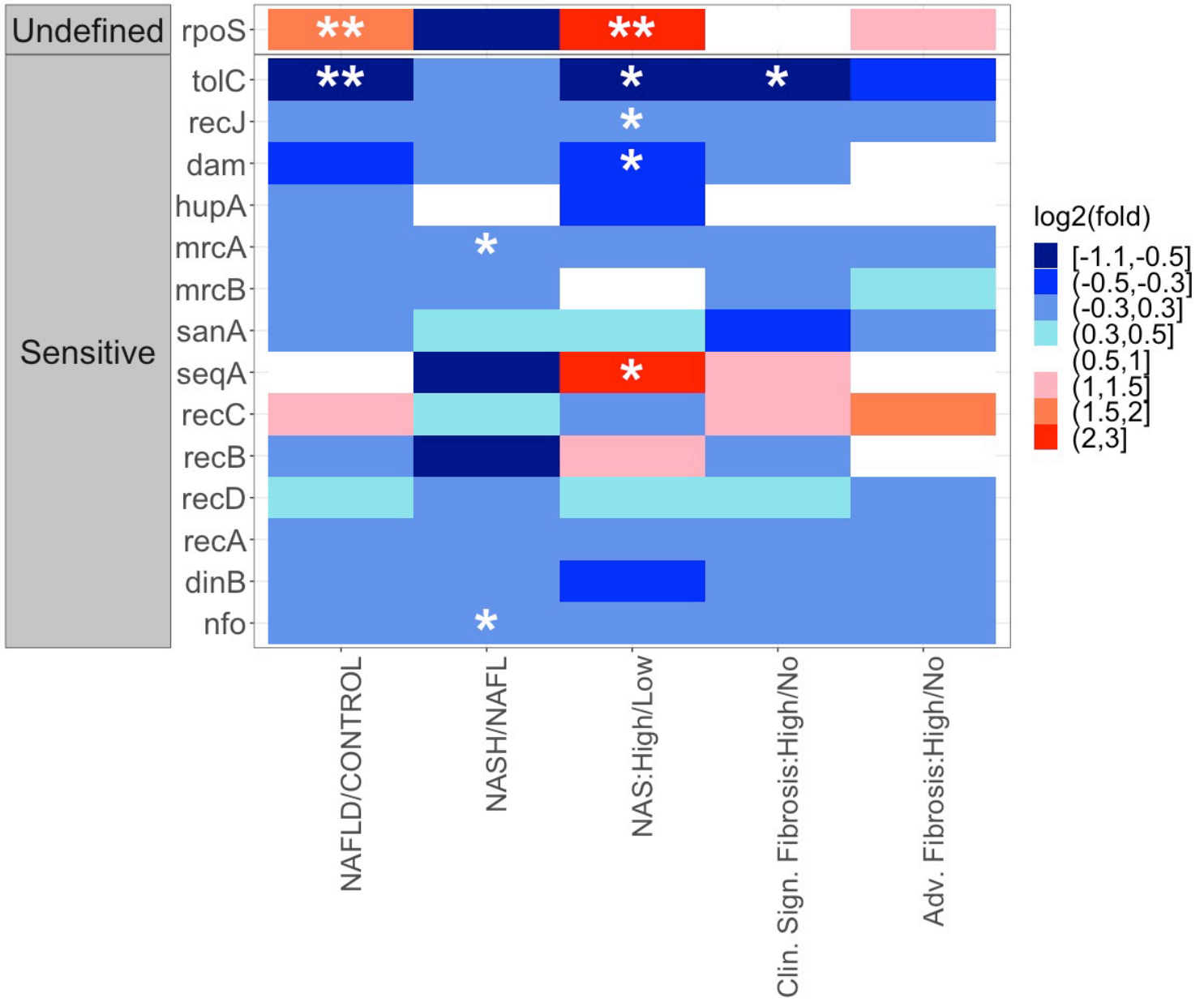


Heatmap of the average log2 fold change.

*Significant taxa are denoted by **

BSH: bile acid hydrolase;
baiCDH: bile acid operon.

General suppression of microbial genes that are in taxa sensitive to the antibiotic effects of bile acids



Significance

- Preliminary data demonstrate a strong relationship between circulating deoxycholate (DCA) and its keto derivatives especially 12-keto-lithocholate with increasing fibrosis severity and clinical decompensation in MASH
- DCA and lithocholate (LCA) are well known to be cytotoxic and DCA promotes oncogenesis and stellate cell collagen production.
- However, the sample size is small and need validation in larger cohort studies

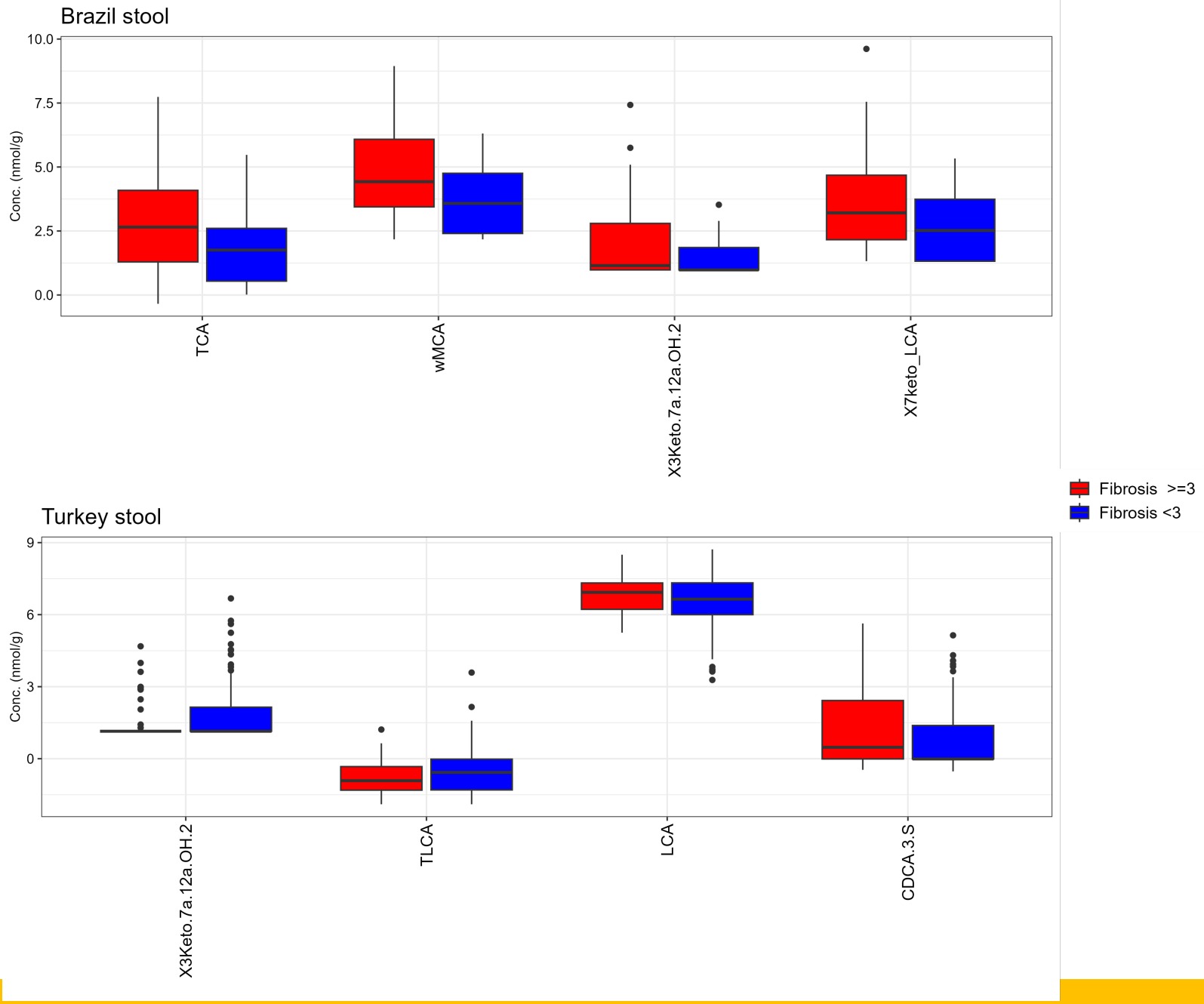
Results: validation data

A global cohort of MASLD patients from Türkiye, Brazil and Singapore

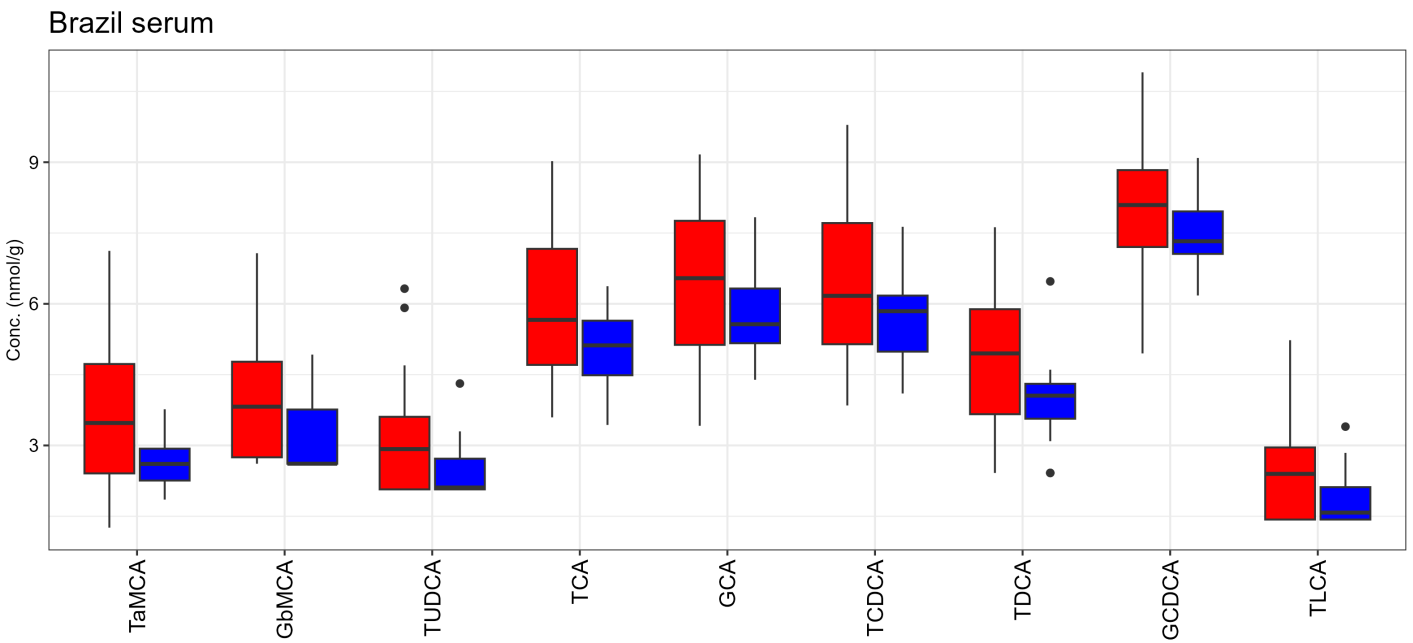
variable	level	Fibrosis <3	Fibrosis >=3	p
n		169	216	
Site (%)	Brazil	58 (34.3)	20 (9.3)	<0.001
	Singapore	29 (17.2)	53 (24.5)	
	Turkey	82 (48.5)	143 (66.2)	
Age (mean (SD))		56.11 (10.77)	46.54 (11.91)	<0.001
Gender (%)	Female	95 (56.2)	94 (43.5)	0.018
	Male	74 (43.8)	122 (56.5)	
bmi (mean (SD))		34.54 (24.20)	33.16 (5.98)	0.422
fibrosis.f (%)	0	0 (0.0)	54 (25.0)	<0.001
	1	0 (0.0)	89 (41.2)	
	2	0 (0.0)	72 (33.3)	
	2.5	0 (0.0)	1 (0.5)	
	3	112 (66.3)	0 (0.0)	
	3.5	1 (0.6)	0 (0.0)	
	4	56 (33.1)	0 (0.0)	

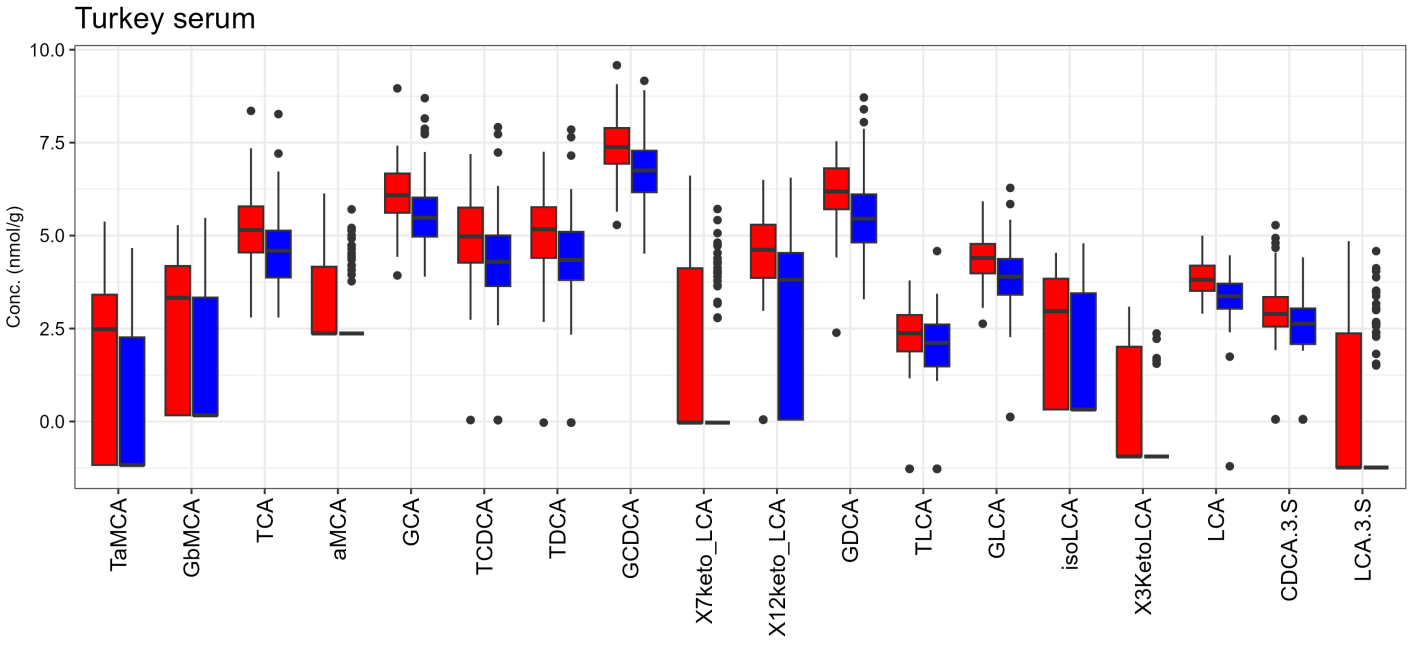
Available patient data

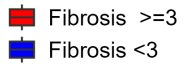
- Türkiye
  - Stool and serum BA
  - 16S microbiome for all patients
- Brazil
  - Stool and serum BA
  - 16S microbiome for a subset of patients
- Singapore
  - 16S microbiome



- LCA consistent signature for increase in advanced fibrosis (our data and literature)
- Confirmed decrease in TLCA (literature)
- Observed increase in TCA, the pre-cursor of DCA (confirmed serum signature reported in literature)



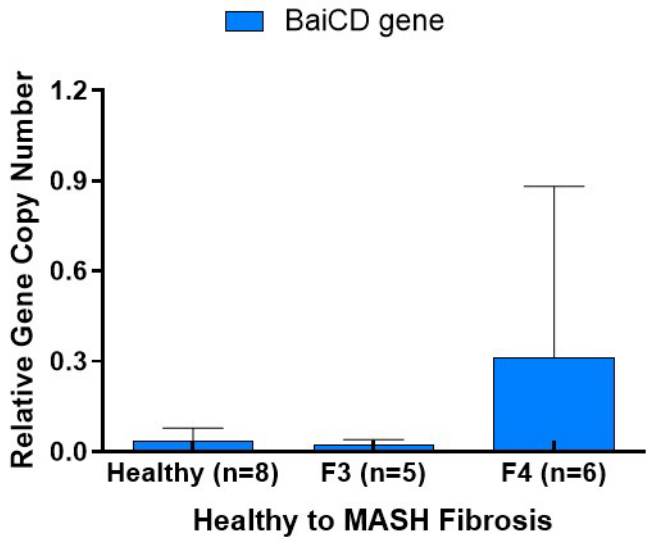




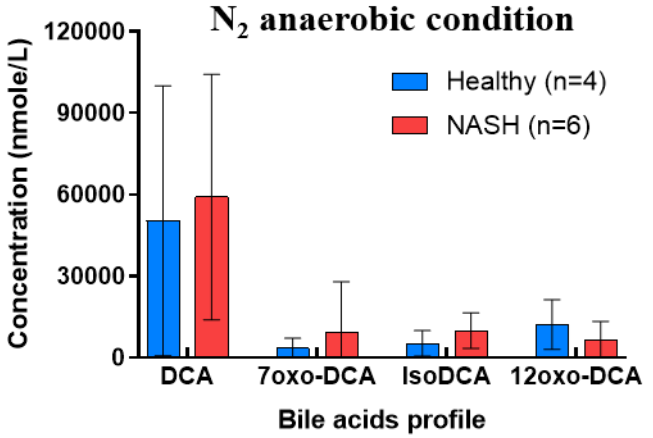
- Brazil and Türkiye: confirm an increase in DCA species (TDCA, GDCA)
- Brazil: confirmed increase in TUDCA
- LCA and 12-keto-LCA were increased. These confirm increased DCA and metabolites.
- Türkiye: sulfated bile acids CDCA-3S and LCA-3S were also increased

Mechanistic studies by qPCR

- BaiCD gene dosage, measured by qPCR in stool of healthy vs MASLD patients
- baiCD expression was increased with advanced fibrosis, particularly in cirrhosis



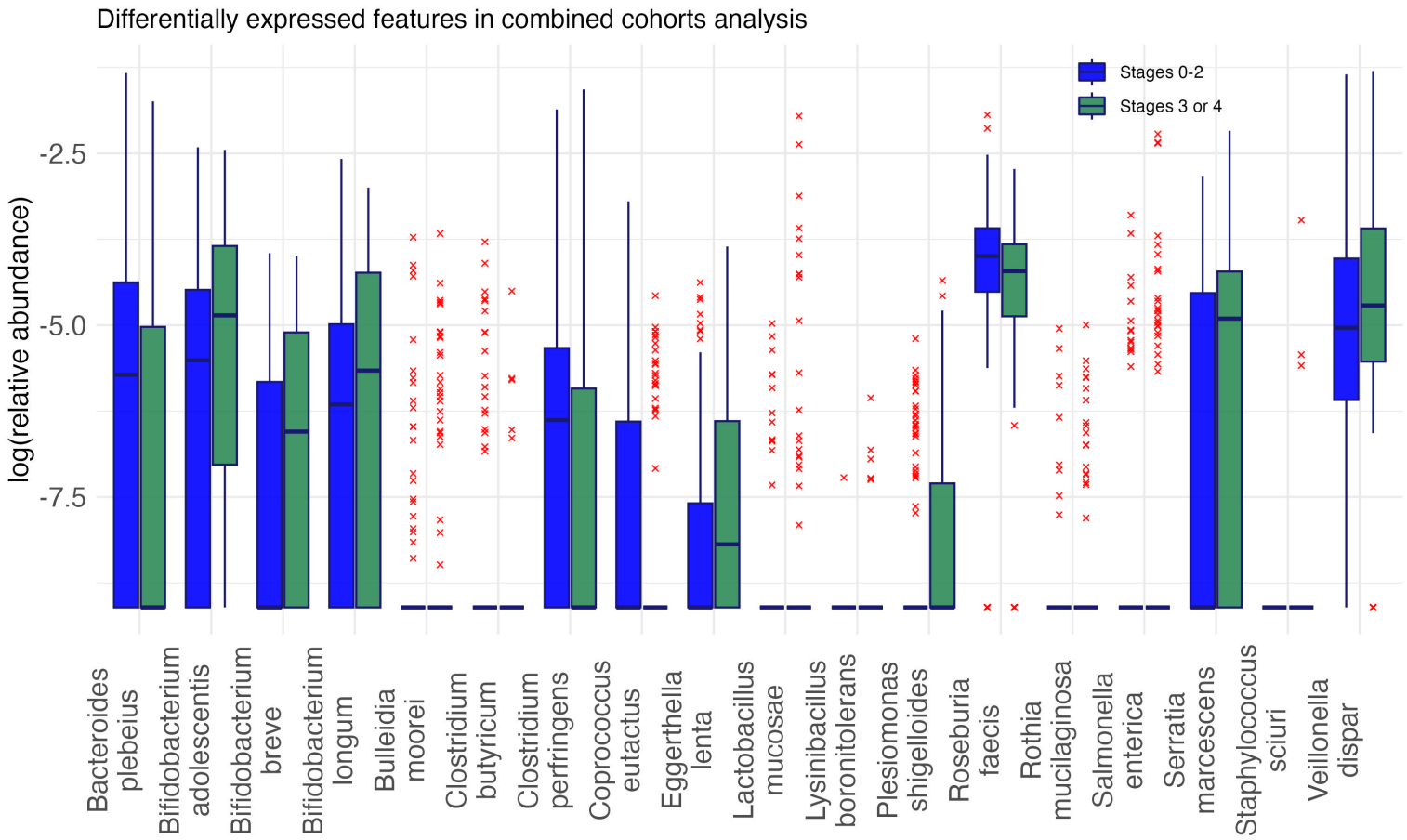
- Bile acid metabolites produced from CA by fecal samples
- DCA, 7-oxo-DCA, isoDCA, 12-oxo-DCA were all produced in excess in MASH with advanced fibrosis compared to healthy controls



Summary: bile acid analysis

- Alternations in serum and gut bile acids have been linked to MASH and to fibrosis
- DCA and its derivatives were identified as a signature for fibrosis progression
- Increasing liver fibrosis is associated with greater DCA and its derivatives as well as serum conjugated cholic acid in a Turkish cohort, confirming the generalizability of the DCA signature associated with increased fibrosis
- Generation of DCA metabolites are increased in MASH with increased fibrosis and related to an increase in the gut bacteria bai operon responsible for DCA synthesis from cholic acid
- We demonstrated generalizability of DCA signature developed on Western populations to Middle East (Türkiye)

Microbiome activity



While several species were common only
Eggerthella lenta was both increased in VCU pilot study and multi-ethnic confirmatory cohort and had bile acid metabolizing activity.

Summary: microbiome analysis

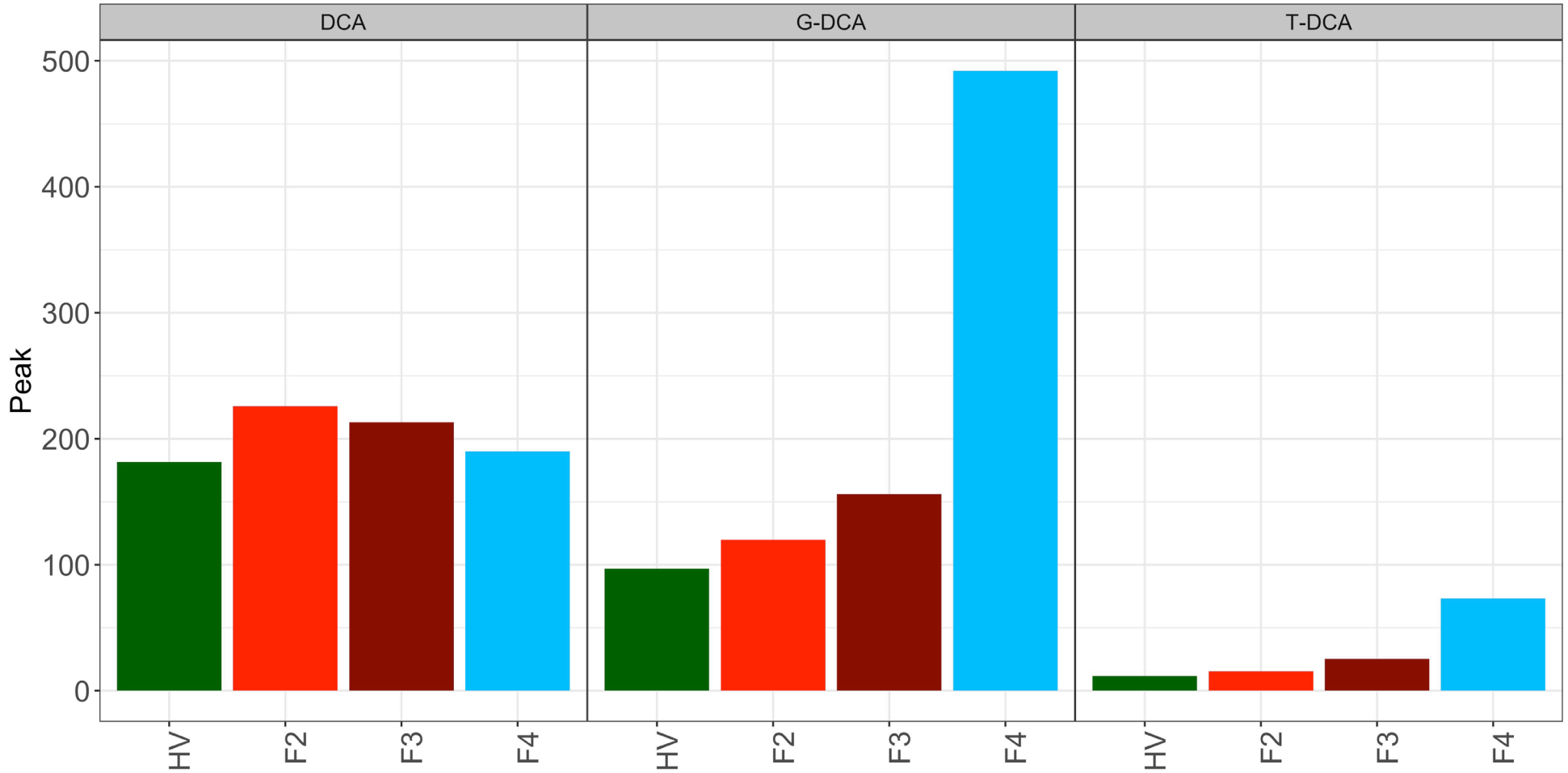
- Existing literature identified microbial signatures of advanced fibrosis mostly in Western and in Asian cohorts; however these analyses were performed under heterogeneous conditions
- Current study comprehensively analyzed a diverse population from Türkiye, Singapore and Brazil under same sequencing conditions
- A common set of microbial signatures for increasing fibrosis was identified
- These support the development of a gut microbiome-based biomarker profile for advanced fibrosis and provide a rationale for targeted mechanistic studies

Related work and future directions

- MASLD is a heterogeneous disease characterized by both progression and regression
- Current work concentrated on one timepoint: need to conduct longitudinal studies to connect the changes in bile acid to disease evolution
- Mechanistic studies are limited to a very small sample size: need to extend to confirmatory studies
- Diet plays an important role in microbiome composition: need to collect a detailed diet survey at microbiome data collection

Backup

Serum BA levels are associated with NASH fibrosis stage



DCA derivatives versus fibrosis change (% from baseline) in placebo arm from ATLAS study

- Fibrosis improvement responders had numerically greater reduction in DCA derivatives